



Possible Long-Term Health Effects of Short-Term Exposure to Chemical Agents

**Volume I
Anticholinesterases and Anticholinergics**

**POSSIBLE LONG-TERM HEALTH EFFECTS
OF SHORT-TERM EXPOSURE TO CHEMICAL AGENTS**

**Volume 1
Anticholinesterases and Anticholinergics**

prepared by the

**Panel on Anticholinesterase Chemicals
Panel on Anticholinergic Chemicals**

**Committee on Toxicology
Board on Toxicology and Environmental Health Hazards
Assembly of Life Sciences**

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PREFACE

The Department of the Army asked the Committee on Toxicology (COT) of the National Research Council (NRC) Assembly of Life Sciences to conduct a study of the possible chronic adverse health effects on servicemen of experimental exposure to various chemicals at U.S. Army Laboratories at Edgewood, Maryland, in about 1958-1975. The Edgewood tests were conducted to learn how specific chemicals may affect humans. Some 6,720 soldiers took part in the program; 254 chemicals were administered by various routes. Chemicals were tested for various military applications. Among them were acutely toxic anticholinesterase chemicals; incapacitating agents, which included the glycolates, atropine-like anticholinergic compounds of which BZ (3-quinuclidinyl benzilate) is a prototype; the indoles, represented by EA 1729 (LSD-25); the cannabinoids, or marijuana-like compounds; and the sedative, or "tranquilizer," group.

Review of the Edgewood tests included interviews with key administrators, investigators, nurses, and technicians. The account of practices and procedures undertaken at Edgewood comes from a wide spectrum of sources, both documents and interviews. They represent a wide spectrum of attitudes, but are essentially in agreement. Committees were formed at Edgewood to review classified chemicals and reports for declassification and use by NRC panels. Extensive extracts were prepared of preclinical animal and human protocols and technical reports at Edgewood Libraries and other Edgewood facilities where records of subjects and details of exposure conditions and clinical findings are maintained. A repository was established at Edgewood Arsenal (August 1980) for storing the reports and information obtained from other sources.

The NRC staff organized the test chemicals into several pharmacologic classes; the first two classes consisted of 15 anticholinesterase (Appendix A) and 24 anticholinergic (Appendix B) chemicals. Two expert panels were established (on anticholinesterases and on anticholinergics) with chairmen selected from the COT and members drawn from the scientific community on the basis of familiarity with some aspect of the pharmacologic class involved or expertise in a discipline needed for proper evaluation of potential adverse health effects.

Research and experimental case files on volunteers were extracted and summarized. Digests of the literature were prepared.

The charge of the two panels was to determine whether, on the basis of the data available, it is possible to demonstrate the likelihood of long-term health effects or delayed sequelae and, if so, whether the involved chemicals, as tested, are likely to produce long-term health effects or delayed sequelae. The charge did not include policy recommendations regarding human testing. This report is the first of two on the Edgewood tests. It presents the two panels' tentative conclusions which are based on a review of data obtained from Edgewood in nine documents provided by NRC staff (listed in Appendixes C and D), on mortality data organized by the NRC Medical Follow-Up Agency, and on separate discussions and papers generated

by the panel members. Specific issues addressed were based on this information and relevant items from the published literature.

A second report will contain an evaluation of most of the remaining chemicals tested at Edgewood and an evaluation of the effects on morbidity state of the test subjects. The information on morbidity may permit firmer conclusions than are presented here.

EXECUTIVE SUMMARY

In response to a request from the Department of the Army, the Committee on Toxicology (COT) of the National Research Council Assembly of Life Sciences conducted a study to evaluate the possibility of long-term or delayed adverse health effects of chemical agents tested on military volunteers during the 1960s and 1970s.

The task was begun about 2 years ago, with interviews of key people who had been associated with the soldier-volunteer test program of the Army Chemical Center (Edgewood Arsenal), Maryland. Initial efforts included a thorough review of the Army's laboratory and clinical records and of reports in the scientific literature. Some 6,720 soldiers took part in the Edgewood program as test subjects in about 1958-1975, and 254 chemicals were administered in an experimental setting.

The chemicals were divided into eight major pharmacologic classes and organized within each class according to structure. The most extensively studied classes are the anticholinergic and the anticholinesterase chemicals, and these are the subjects of this report; the other classes will be reported on later. Panels were then formed to study these two main classes. The chairmen were selected from the COT, and the members were selected for expertise in some aspect of the review of the pharmacologic class in question.

The anticholinesterases are generally organophosphates; these are nerve agents resembling parathion. Major symptoms of low-level anticholinesterase exposure include salivation, increased sweating, contracted pupils, and bronchospasm. The anticholinergics are generally "glycolates", (substituted glycolic and tropic acid esters) of which the representative and best-known member is atropine. Major symptoms of low-level atropinization include dry mouth, dilated pupils, and tachycardia. There were 24 anticholinergics tested on about 1,800 subjects. There were 15 anticholinesterases tested on about 1,400 subjects. These two classes are readily paired, in that members of each are used as treatment for overexposure to members of the other.

The next step involved organization of Edgewood data and reports. Some of this material had to be declassified before use by the panels. Digests of the entire available literature, classified and unclassified, were prepared by consultant pharmacologists, and various documents were made available to the panels for their use in investigating the possibility that the Edgewood test experience resulted in persistent adverse effects. The panels met several times, beginning in June 1980.

The specific charge to the two panels was to determine:

- o Whether the data available are sufficient to estimate the likelihood that the test chemicals have long-term health effects or delayed sequelae.
- o Whether the involved chemicals, as tested, are likely to produce long-term adverse health effects or delayed sequelae in the test subjects.

ANTICHOLINESTERASE CHEMICALS

The panel concludes that although no evidence has been developed (to date) that any of the anticholinesterase test compounds surveyed carries long-range adverse human health effects in the doses used, the results of an ongoing NAS/NRC morbidity study may shed further light on this issue. The panel therefore is unable to rule out the possibility that some anti-ChE agents produced long-term adverse health effects in some individuals. Exposures to low doses of OP compounds have been reported (but not confirmed) to produce subtle changes in EEG, sleep pattern, and behavior that persist for at least a year. Whether the subjects at Edgewood incurred these changes and to what extent they might now show these effects are not known. If such changes occurred and persisted, they would be difficult to detect now. They could be determined scientifically only by a new study in which EEG, sleep state, and psychologic-test scores were compared with those from nonexposed control subjects. This might be considered, if reasonable suspicion develops, based on responses obtained in the referenced morbidity study, that selected subjects experienced behavioral changes traceable in onset to experimental exposure to the anti-ChE agents.

ANTICHOLINERGIC CHEMICALS

No firm evidence has been seen that any of the anticholinergic test compounds surveyed produced long-range adverse human health effects in the doses used at Edgewood Arsenal. More intensive study is required to confirm this conclusion. The high frequency of uncontrolled variables makes evaluation of behavioral effects difficult.

On the basis of available data, in the judgment of the panel, it is unlikely that administration of these anticholinergic compounds will have long-term toxicity effects or delayed sequelae. An ongoing morbidity study should provide more definitive information once it is completed.

MORTALITY

Standardized mortality ratios were derived from mortality data for the soldiers (all males) who participated in the Edgewood Tests and from U.S. mortality rates. For each class of chemicals, the mortality rates among the soldiers were not significantly higher than the rates of the U.S. population, categorized by age and calendar year.

MORBIDITY

An ongoing morbidity study among the test subjects is expected to provide a more complete understanding of the long-term consequences of exposure to anticholinergic and anticholinesterase chemicals.

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INTRODUCTION

HISTORY OF THE EDGEWOOD TESTING PROGRAM

Human experimentation appears to have been an integral part of the history of the U.S. Army chemical warfare (CW) research efforts until its suspension in 1975. On June 28, 1918, the President directed the establishment of the Chemical Warfare Service (CWS). Four years later, in October 1922, the CWS created a Medical Research Division to conduct research directed at providing a defense against chemical agents. No matter how exhaustively an agent was tested in animals, it was felt that its efficacy in humans also had to be studied.

In early 1941, the threat of war increased the urgency of the development of protection against CW agents and, consequently, engendered a need for a larger source of volunteers. Formal authority to recruit and use volunteer subjects in CW experiments was initiated in 1942. The Secretary of War was asked to rule on the permissibility of using enlisted men for testing agents of the mustard-gas type. In July 1943, the CWS was assigned responsibility for all medical research related to CW. This extension of the CWS mission included toxicologic research and the study of hazards to the health of personnel in the CWS.

The issue of the use of human volunteers was considered by the Armed Forces Medical Policy Council during the early 1950's. The Council concluded that essential data could not be obtained unless human volunteers were used, and the use of humans in medical research was authorized. By 1954, the Chemical Corps (formerly CWS) had established a framework within which to conduct human experimentation, but it lacked an adequate pool of volunteers. In 1955, it was decided that the most practical source of volunteers would be enlisted men stationed at Army installations in the vicinity of Edgewood Arsenal. It was emphasized that voluntary consent of each human subject was absolutely essential. It was also stated that, in all experiments involving volunteer subjects, the subjects would be thoroughly informed of all procedures and of what might be expected as a result of each test. Furthermore, each volunteer would be free to determine whether he desired to participate in a given experiment. In October 1959, approval was granted for the conduct of research on volunteers to investigate defense against incapacitating CW agents.

The search for incapacitating agents intensified when the Kennedy administration took office. The involvement with incapacitating agents represented a departure from an earlier period, begun in 1946, when interest in highly toxic (acute) anticholinesterase chemicals resulted from their development in Germany during World War II. The basic purpose of a military incapacitating agent is to produce temporary ineffectiveness without permanent injury or death. Incapacitating agents (anticholinergic chemicals) and highly toxic (acute) anticholinesterase chemicals produce functional and structural effects on the nervous system which cause rapid or delayed effects on an individual's performance and behavior.

PROCEDURES USED AT THE EDGEWOOD CHEMICAL TESTING PROGRAM

A fairly extensive discussion of the procedures used is provided in the Inspector General's report, Use of Volunteers in chemical Agent Research, prepared by Colonel James R. Taylor and Major William H. Johnson and dated March 1976 (listed in Appendixes C,D).

RECRUITMENT OF VOLUNTEERS

Recruiting teams (initially administrative officers, but later often including military physicians from the Edgewood laboratory) visited Army installations where a briefing, usually with a film and handouts, was presented to a large number of enlisted men. Generally 10 to 20 percent of the audience expressed interest and these men were asked to complete a personal history, which included medical and psychologic items and the Minnesota Multiphasic Personality Inventory (MMPI). It was not unusual for 400-600 men to request assignment in the course of a tour of seven to ten installations. Of these, no more than 100 were selected and eventually assigned for a 1- to 2- month period of temporary duty at Edgewood Arsenal.

The "incentives" for volunteering consisted of a small monetary allowance (approximately \$1.50 a day for temporary duty), the assignment of only light duties while at Edgewood, and almost every weekend free. Some volunteers were genuinely interested in the scientific and experimental aspects; however, if curiosity or the desire to "test one's self" seemed too strong, the applicant was usually not accepted.

As a group, the volunteers were above average in physical and mental qualifications, with a mean IQ near 110, good behavior records, and "normal" MMPIs with profiles generally within two standard deviations of the population mean on all scales.

GUIDELINES FOLLOWED IN THE PROTECTION OF SUBJECTS

The Nuremberg and Helsinki guidelines were regarded by the investigators and their supervisors as appropriate constraints in studies performed on volunteers, although this was not clearly articulated in official memoranda until the mid-1960s. The provision of accurate, informative explanations of what was planned and what might be expected was regarded as essential to the continuance of the program. Written consents, witnessed by medical staff members, were required from the outset and became more elaborate with time. However, minutes of hearings conducted by the U.S. Senate Subcommittee on Health and Subcommittee on Administrative Practice and Procedure, September 10-12, 1975, stated that the consent information was inadequate by current standards.

INVESTIGATORS

When BZ studies were begun in 1960, the need for a psychiatrist with biologic training and interest was recognized, and one was assigned to the program in January 1961. Physicians trained in internal medicine, anesthesiology, cardiology, surgery, dermatology, ophthalmology, neurology, and other specialties were assigned as the program proceeded. Many were research-oriented and have since gained excellent reputations in academic medicine at leading universities.

SELECTION OF DOSES FOR HUMAN TESTS

Subthreshold doses based on estimates from animal potency studies were used in the first few subjects. For example, the earliest exposures to BZ, one of the anticholinergic test compounds, were at doses between 0.1 and 0.5 $\mu\text{g}/\text{kg}$, which was less than one-tenth the incapacitating dose (ID) ultimately established at approximately 5.5 $\mu\text{g}/\text{kg}$. The intravenous route was preferred initially, but other routes of administration were also used. Inhalation studies were sometimes undertaken after a compound had been thoroughly studied by one of these parenteral routes. Oral and percutaneous studies were performed when effectiveness via these routes was of interest.

As the program developed, it became customary to test agents at dose increments of 40 percent, once the approximate effects of the lower doses were known. Placebos were used in some studies, but the cost with respect to subject confinement time, staff workload, and delay in achieving estimates of potency made this impractical except in special cases (e.g., evaluation of antagonists). Instead, low and high doses were assigned in a randomized manner by someone not involved in an experiment. Placebo responses were minimal. Signs of drug effects at all but the lowest doses were significant and made the value of placebo or "no treatment" inconsequential.

RANGE OF DOSES

Rarely did the intramuscular or intravenous doses exceed 1.5 times the incapacitating dose. Inhalation doses were higher, but potencies were lower by this route (usually about 60 percent of that by the intravenous or intramuscular route). Compared with doses described in the scientific literature on atropine coma therapy (18-23) or scopolamine therapy (19), the BZ doses to which volunteers were exposed appear modest. As much as 20 times the ID_{50} of atropine and 30-40 times the ID_{50} of scopolamine have been administered in the past by clinicians--often to older and less robust patients. Many patients received multiple exposures of this magnitude over a period of days or weeks. These therapeutic procedures, reported several decades ago in refereed journals, actually stressed and advocated the benefits of such treatment, despite occasional deaths (most of which appear to have been caused by hyperthermia).

SAFETY MARGIN

The safety margin of a drug is defined as the ratio of the lethal dose (LD) to the effective dose (ED). Sometimes, ratio of the LD_{50} to ED_{50} is used, although a more conservative approach favors the use of the ratio of LD_1 to ED_{99} (standard margin of safety). In the case of incapacitating agents, much reliance is placed on extrapolation from animal experimentation, and estimation of the LD_1 is generally unreliable.

Many other extrapolation techniques have been used in manipulation of animal lethality data in an effort to generate a reasonable human estimate. By taking a conservative approach with data on deaths at low doses, one can derive estimates for man that are modest and in keeping with clinical judgement. Such methods depend on procedures developed and applied in toxicology.

ANTICHOLINESTERASES

Anticholinesterases (anti-ChEs) are toxic to humans principally because they interfere with molecular and cellular mechanisms required for the normal functioning of the central nervous system (CNS) and peripheral nervous system (PNS). Their adverse health effects are related mostly to inhibition of acetylcholinesterase (AChE), a critically important CNS and PNS enzyme that hydrolyzes the neurotransmitter acetylcholine (ACh). Chemical-warfare (CW) agents exploit the acute, life-threatening properties of profound AChE inhibition; some of the anti-ChEs precipitate other clinically significant deleterious effects on sensory and neuromuscular function. All the major anti-ChEs are chemically reactive and potentially capable of alkylating a variety of biologic macromolecules, but the long-term health implications of these reactions are not well known. This chapter therefore focuses on the acute toxic effects of anti-ChEs and the possible long-term effects on CNS and PNS function.

The fundamental cellular component of the nervous system, the neuron, has long, branching, cylindric processes (dendrites and axon) that extend from the cell body. Dendrites are modified for signal reception and transduction and form extensive networks that permit interneuronal communication, coordination, and integration of nervous-system function. The axon typically is a long extension of the neuron specialized for transmission of electric signals and, at its distal end, for chemical communication of information to other neurons or to muscle at sites termed synapses and neuromuscular junctions, respectively.

ACh is the chemical transmitter of information from both somatic and autonomic (PNS) neurons. On the somatic side, the lower motor neuron uses ACh to convey excitatory impulses to voluntary muscle. Cholinergic neurons of the autonomic division of the PNS are grouped in craniosacral (parasympathetic) and thoracolumbar (sympathetic) outflows from the spinal cord. Parasympathetic pathways use ACh at both preganglionic and postganglionic neurons; in the sympathetic system, ACh is restricted to the preganglionic effector. These cholinergic-dependent autonomic neurons play an important role in regulating the function of various vitally important effector organs. Cholinergic pathways are widely distributed in CNS tissue, but their functions are less well understood than those in the PNS.

AChE terminates the transmitter action of ACh. Drugs that inhibit or inactivate AChE (anti-ChE agents) cause ACh to accumulate at cholinergic sites and thus produce effects equivalent to continuous stimulation of cholinergic nerve fibers. Before World War II, only "reversible" anti-ChE agents were generally known, of which physostigmine (eserine) is the outstanding example. Shortly before and during World War II, a class of highly toxic chemicals, the organophosphorus compounds (OPs), were developed, chiefly by Schrader of I.G. Farbenindustrie, first as agricultural insecticides and later as CW agents. The high potency of these compounds was found to be due to "irreversible" inhibition of AChE; thus, they produced effects for considerably longer periods than the classical

inhibitors. Since the pharmacologic actions of both classes of anti-ChE agents are qualitatively similar, they are discussed as a group, and important features of individual classes or compounds are noted.

CHEMISTRY OF ANTICHOLINESTERASES

A complete list of anti-ChE compounds used in the Edgewood program is contained in the master file (Appendix A). Structure-activity relationships have been reviewed extensively for the "reversible" inhibitors (1,2) the OP agents (3,4) and both classes of compounds (5-7).

"REVERSIBLE" INHIBITORS

After the structure of physostigmine was established, Stedman (8,9) undertook a systematic investigation of a number of related synthetic compounds. The essential moiety of the physostigmine molecule was found to be the methylcarbamate of a basically substituted, simple *o*-aminophenol. The quaternary ammonium derivative, neostigmine, is a compound of greater stability and equal or greater potency. Retention of the dimethylcarbamate side chain in the meta position, but with incorporation of the quaternary nitrogen atom into the ring to form a pyridyl nucleus, results in compounds with anti-ChE and other pharmacologic properties similar to those of neostigmine. Pyridostigmine is a drug of this class.

Although the carbamates are the most familiar and the most commonly encountered anti-ChEs, members of other chemical classes are also capable of inhibiting AChE. For example, edrophonium, an analogue of the phenolic residue of neostigmine, is used extensively in clinical medicine. It was not administered to the volunteers at Edgewood, but two other noncarbamates were:

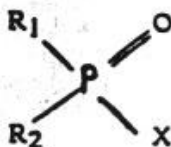
1,2,3,4-tetrahydro-9-acridinamine (Tacrine) and hexafluorenum (Mylaxen). These compounds also are used in clinical medicine but are not as popular as neostigmine, pyridostigmine, or edrophonium.

ORGANOPHOSPHORUS (OP) INHIBITORS

The general formula for this class of cholinesterase inhibitors is shown in Figure 1. A great variety of substituents is possible: R₁ and R₂ may be alkyl, alkoxy, aryloxy, amido, mercapto, or other groups; and X (also called the "leaving group") may be a halide, cyanide, thiocyanate, phenoxy, thiophenoxy, phosphate, alkylthioethylmercaptide, dialkylaminoethylmercaptide, or carboxylate group. It is obviously impossible to discuss here more than a few representative compounds of the more than 50,000 that

Fig. 1: Organophosphorus Compounds

General Formula:



have been prepared; a useful chemical classification of the compounds in this class that are of particular pharmacologic or toxicologic interest has been developed by Holmstedt (3,4). Diisopropyl phosphorofluoridate (DFP) is perhaps the best known and the most extensively studied compound.

Generally, the most acutely toxic compounds are those with one carbon-phosphorus bond (phosphonates). The compounds studied by the Army (Appendix A), with the exception of DFP, contain this feature. In GA (tabun), the leaving group is cyanide. In GB (sarin), GD (soman), and GF, the leaving group is fluoride. In the V agents, VX and EA 3148 (the most potent agent administered to the volunteers), the leaving group is a dialkylaminoalkylmercaptide.

ABSORPTION, FATE, AND EXCRETION OF ANTI-CHES

Physostigmine is readily absorbed from the gastrointestinal tract, subcutaneous tissues, and mucous membranes. It undergoes hydrolytic cleavage at the ester linkage by cholinesterases; renal excretion plays only a minor role in its disposal. In man, a 1-mg dose of physostigmine injected subcutaneously is largely destroyed in 2 h. Neostigmine and related quaternary ammonium drugs are absorbed poorly after oral administration, and much larger doses are needed for effect than when they are administered by injection; both are metabolized by hepatic microsomal enzymes (10) and excreted in the urine.

The commonly encountered OP anti-ChE agents are, with some exceptions (e.g., echothiophate), highly soluble in lipids. Consequently, they are rapidly and effectively absorbed when administered by almost any route, including the gastrointestinal tract, the skin and mucous membranes after contact with the liquid form, and the lungs after inhalation of vapors, finely dispersed dust, or aerosols. Most OP compounds are excreted almost entirely as metabolites in urine. Between the time of absorption and excretion, there are varied periods during which the original compound or its metabolites remain bound to proteins in the blood and tissues.

Both hydrolytic and oxidative enzymes are involved in metabolism of the OP compounds. The OP anti-ChE agents are hydrolyzed in the body by a group of enzymes, the phosphorylphosphatases. These are widely distributed and hydrolyze a large number of OP compounds (e.g., DFP, tabun, sarin, paraoxon, and tetraethyl pyrophosphate, or TEPP) by splitting the anhydride-like P-F (or P-CN) bond. They also hydrolyze several aliphatic esters (e.g., ethyl acetate) and aromatic esters (e.g., phenyl acetate). The enzymes are not irreversibly inhibited by OP compounds, presumably because the phosphorylated active site reacts rapidly with water to regenerate the free form, in contrast with its high stability in the case of the cholinesterases.

Because of the above reactions, the effects of exposure to two OP insecticides may be synergistic. For example, when malathion is administered to animals in combination with *O*-ethyl *O*-*p*-nitrophenyl phenylphosphonothionate (EPN), the resulting toxicity is as much as 50 times that expected from the sum of their individual toxicities, this results primarily from the inhibition by EPN of enzyme systems that normally metabolize malathion to inactive products. Other combinations of OP insecticides also have shown supra-addition of toxic effects.

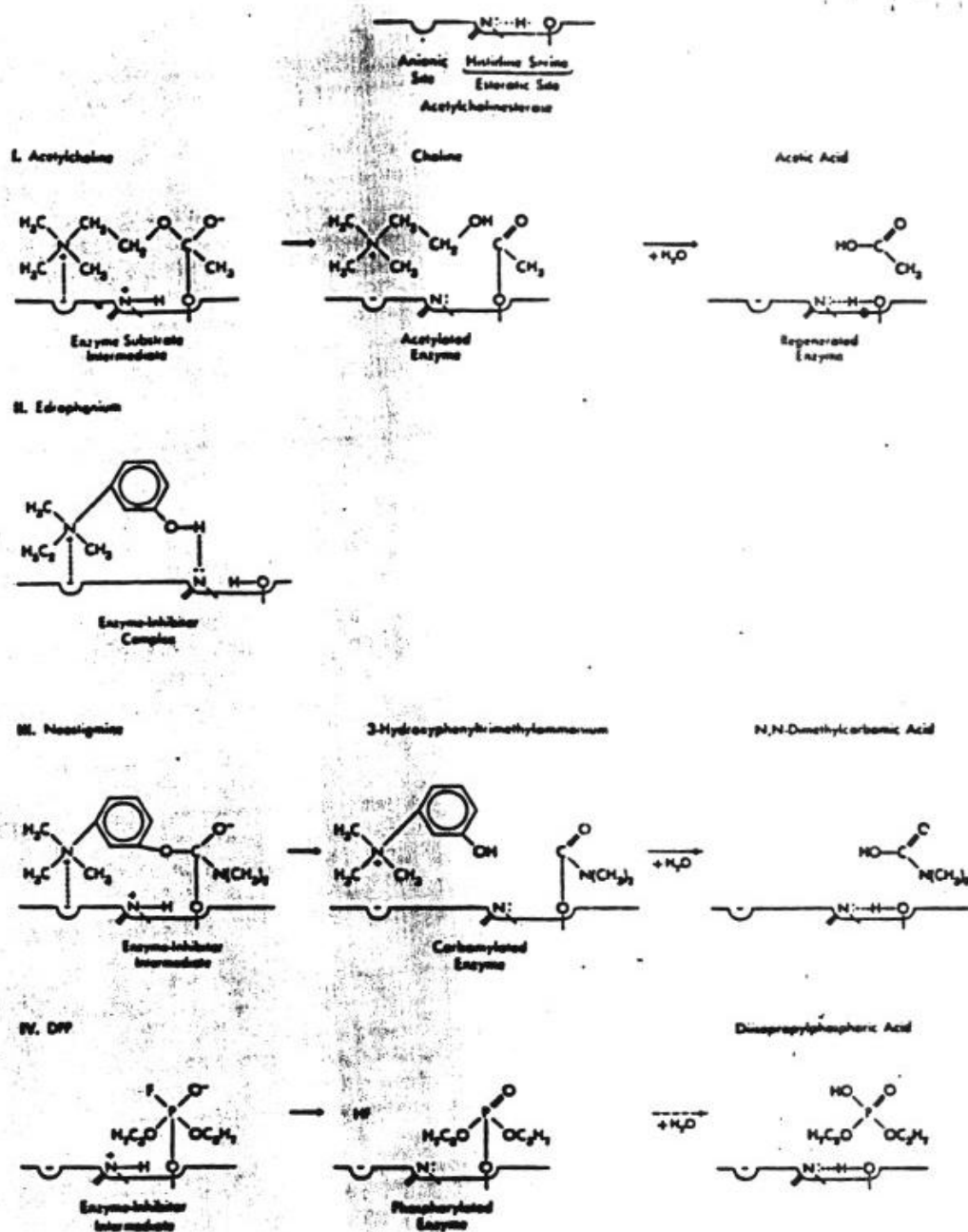


Fig. 2: Steps involved in the hydrolysis of acetylcholine (ACh) by acetylcholinesterase (AChE) (I), and in the inhibition of AChE by reversible (II), carbamyl ester (III), and organophosphorus (IV) agents. Heavy, light, and dashed arrows represent extremely rapid, intermediate, and extremely slow or insignificant reactions, respectively. Reproduced from Koelle, G.B., In: *The Pharmacologic Basis of Therapeutics* (Goodman, L.S. and Gilman, A., eds.), 5th ed. Macmillan Publ. Co., 1975, pg. 448.

MECHANISMS OF ACTION

INHIBITION OF ACETYLCHOLINESTERASE BY ANTICHOLINESTERASES

The mechanisms of action of compounds that typify the three classes of anti-ChE agents are shown in Figure 2. They differ primarily in quantitative respects from the reaction between AChE and its normal substrate, ACh.

Simple quaternary compounds, such as edrophonium, form electrostatic bonds with the anionic site of the enzyme and hydrogen bonds with the imidazole nitrogen atom of the esteratic site. In all such cases, inhibition is rapidly reversible, and such drugs have a very short duration of inhibitory action.

It was at one time generally assumed that physostigmine, neostigmine, and related inhibitors that possess a carbamyl ester linkage or urethane structure, in addition to a tertiary amino or quaternary ammonium group, inhibit the enzyme in the same reversible fashion. However, careful kinetic studies showed that physostigmine and neostigmine are hydrolyzed by cholinesterase (11-14). Initially, inhibitors of this class form complexes in which the inhibitor is attached to the enzyme at both anionic and esteratic sites; subsequently, hydrolysis proceeds in a manner analogous to that of ACh, and the alcoholic moiety is split off, leaving a carbamylated and inhibited enzyme. Later, this enzyme reacts with water to release a substituted carbamic acid and the regenerated enzyme. The main difference between the reaction of the natural substrate, ACh, and that of the carbamate inhibitors is the velocity of the final step; the half-life of dimethylcarbamyl AChE, formed by the reaction with neostigmine, is more than 40 million times that of the acetylated enzyme: 30 min and 42 μ s, respectively (15).

The reaction between AChE and most OP inhibitors, such as DFP, occurs only at the esteratic site. It proceeds in a comparable fashion, except that, as a result of the initial hydrolysis, the enzyme becomes phosphorylated. The resulting phosphorylated enzyme is extremely stable: if the attached alkyl groups are methyl or ethyl, substantial regeneration of the enzyme by hydrolytic cleavage requires several hours; with isopropyl groups, as in DFP, virtually no hydrolysis occurs, and the return of AChE activity depends on synthesis of new enzyme, which requires days to months. Some quaternary OP compounds (e.g., echothiophate) combine at both the esteratic and anionic sites, and that probably contributes to their extreme potency and specificity (4,16).

From the foregoing account, it is apparent that the terms "reversible" and "irreversible," as applied to the carbamyl ester and OP anti-ChE agents, respectively, reflect only quantitative differences and that both classes of drugs react with the enzyme in essentially the same manner as does ACh.

REACTIVATION OF AChE

Although the phosphorylated esteratic site of AChE undergoes hydrolytic regeneration at a low or negligible rate, Wilson (17) found that the nucleophilic agent hydroxylamine (H_2NOH) can reactivate the enzyme much more rapidly. In the subsequent search for more effective reactivators, a large number of hydroxamic acids

(RCONHOH) and oximes (RCH=NOH) was shown to have this property, e.g., diacetyl monoxime, or DAM. From the data that accrued, it was predicted that highly effective reactivation should be produced by a molecule containing both a quaternary nitrogen atom and an oxime group, spaced at an appropriate distance. This goal was achieved (18) with pyridine-2-aldoxime methyl chloride (2-formyl-1-methylpyridinium chloride oxime, pralidoxime); reactivation with this compound occurs in one millionth the time of that with hydroxylamine (19). Some bisquaternary oximes were later shown to be even more potent as reactivators; an example is obidoxime chloride (Figure 3)—1,1'-(oxydimethylene)-bis(4-formylpyridinium), dichloride dioxime (20).

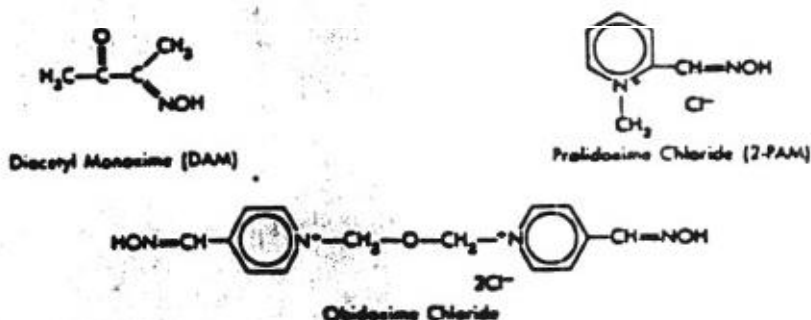


Fig. 3: Cholinesterase reactivators. Reproduced from Koelle, G.B. In: *The Pharmacologic Basis of Therapeutics* (Goodman, L.S. and Gilman, A., eds.), 5th ed. Macmillan Publ. Co., 1975, pg. 458.

The mechanism of reactivation is sketched in Figure 4. When the quaternary ammonium group of pralidoxime is attracted electrostatically to the anionic site of the enzyme, the oxime group of the former is oriented optimally to exert nucleophilic attack on the electrophilic phosphorus atom of the phosphorylated esteratic site; the oxime-phosphonate is then split off, leaving the enzyme (21,22).

Although the oximes reactivate phosphorylated cholinesterase in vitro and increase recovery from intoxication produced in vivo by some organophosphates, they are not panaceas for the treatment of poisoning by anti-ChEs. Pralidoxime and obidoxime are quaternary ammonium compounds, and their capacity to reactivate brain enzymes is inhibited by the blood-brain barrier. It is also debatable whether pralidoxime and related agents can effectively antagonize the manifestations of intoxication by neostigmine and other carbamyl ester inhibitors. Moreover, most phosphorylated AChEs undergo a fairly rapid process termed "aging" and within the course of minutes or hours, thereby become completely resistant to reactivators.

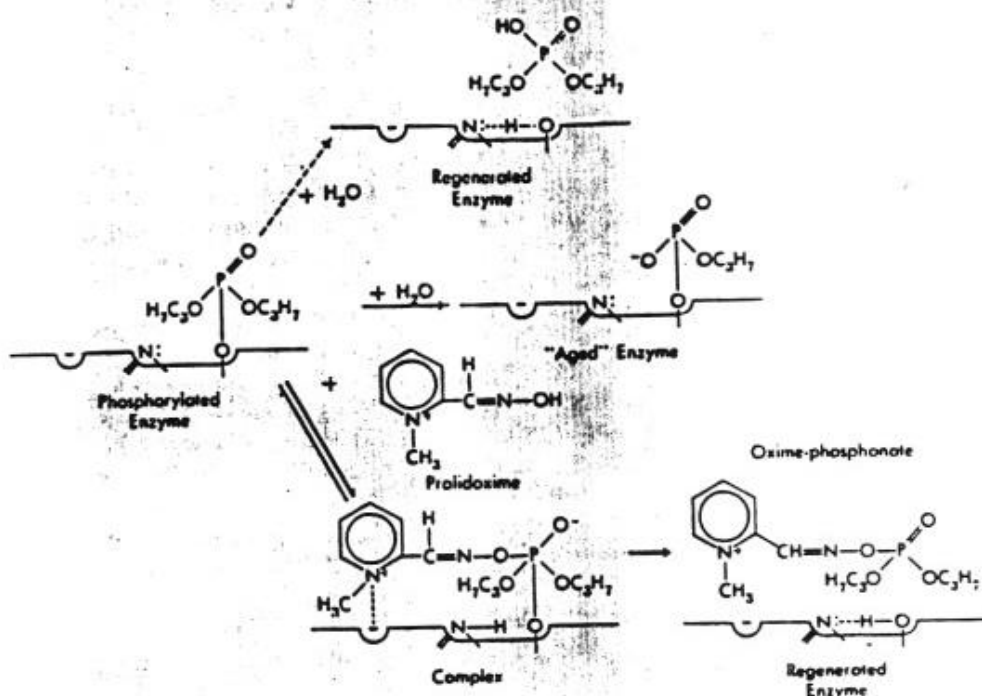


Fig. 4. Reactivation of alkylphosphorylated acetylcholinesterase (AChE). After alkylphosphorylation of AChE by DFP (left), spontaneous hydrolytic reactivation occurs at an insignificant rate (upper reaction), as indicated by the dashed arrow. "Aging" is the loss of one of the isopropoxy residues that occurs more rapidly than spontaneous hydrolysis; the product is very resistant to regeneration by pralidoxime. Pralidoxime (lower reaction) combines with the anionic site by electrostatic attraction of its quaternary nitrogen atom, which orients the nucleophilic oxime group to react with the electrophilic phosphorus atom; the oxime-phosphonate is split off, leaving the regenerated enzyme. Reproduced from Koelle, G.B.: *In The Pharmacologic Basis of Therapeutics* (Goodman, L.S. and Gilman, A., eds.), 5th ed. Macmillan Publ. Co., 1975, pg. 457.

AGING OF INHIBITED ACHE

Aging causes inhibited enzymes to become refractory to reactivation. The phenomenon was first reported in 1955 by Hobbiger (23) and later observed with OP-inhibited AChEs (24,25) and with an OP-inhibited atropinesterase (26). An extensive discussion of aging may be found in Berend's thesis (27).

The process determines the time during which reactivators can be expected to be beneficial for those exposed to OP anti-ChEs. In the case of OP anti-ChEs, an inhibited (e.g., phosphorylated)

enzyme, which initially can be reactivated by oximes, is changed to a form that cannot be reactivated by these compounds (19). The term "aging" has been applied because the amount of inhibited enzyme refractory to reactivation increases with time.

For example, Berry *et al.* (28) observed that, although pretreatment of animals with a combination of pralidoxime (2-PAM) and atropine increased the LD₅₀ values of several compounds (e.g., TEPP and DFP), it had little effect on the lethality of sarin and none on the lethality of soman; they concluded that this was the result of the rapid aging of the ChEs inhibited by sarin and soman. More direct evidence of *in vivo* aging was obtained by Harris *et al.* (29), who injected ³²P-labeled sarin and soman into rats and observed that the rate of aging of the inhibited rat-brain AChE was the same *in vivo* as in parallel *in vitro* experiments.

Aging is probably due to the splitting-off of one alkyl or alkoxy group from the inhibited enzyme, leaving a more stable monoalkyl- or monoalkoxy-phosphoryl AChE (30,31). Wilson (32) pointed out that the difference in reactivatability may be associated with the relative reactivity of secondary and tertiary phosphate esters. Whereas the original inhibited enzyme is a tertiary phosphate ester, the dealkoxylated derivative is a secondary phosphate ester. Thus, the rate of aging depends on the phosphoryl group (33) and not on the group hydrolyzed from the OP by cholinesterase (ChE). Berry and Davies (28) observed, as have many others, that soman yields the most rapidly aged-inhibited ChE obtained from any available anti-ChE; the half-life of the pinacolyl phosphonylated enzyme was determined to be less than 1.5 min. The next most rapidly aged-inhibited enzyme also contained a branched-chain secondary group; (CH₃)₂-CH-CH(CH₃)-OH-. Some straight-chain secondary groups, such as CH₃-CH₂-CH(CH₃)-OH-, were also associated with relatively rapidly aged-inhibited enzymes, the phosphonylated enzyme half-life being about 0.5 h. Berry and Davies (28) noted that aging "is slow when the alkyl group is a primary alcohol, whether or not the carbon chain is branched, but is much more rapid if the alkyl group is a secondary or cyclic alcohol."

CHOLINERGIC RECEPTOR AND ACH CHANNEL

In addition to reacting with ChEs, OPs and other anti-ChEs also can react with other critical molecules in nerves or in effector organs. OP drugs may exert direct effects on the cholinergic receptor or on its phospholipid environment, at both CNS and PNS synapses (34-38). OPs also react with other neural and metabolic enzymes, and some of them are capable of alkylating DNA. The biologic consequences of these reactions are not as well understood as are those inhibiting ChE.

White and Stedman (39) suggested that, in addition to inhibiting AChE, OP compounds have an effect on the site where the ACh molecule reacts at the neuromuscular junction. Riker and Wescoe (40) showed a direct agonist action of neostigmine at the neuromuscular junction, and many others have found that neostigmine and some other anti-ChE agents have antitachycardic effects not apparently related to inhibition of ChE (41). Additional observations indicated that preparations that had been denervated for over 20 d responded with a contracture when exposed to sarin,

and findings described below indicate that anti-ChE agents like neostigmine cause marked destruction of denervated muscle (42,43); this phenomenon is most likely correlated with the partial agonist effect of neostigmine.

Similarly, Miquel (44) suggested that OP compounds react with other sites on the muscle, in addition to the enzyme itself. Studies by Xavier and Valle (45) disclosed that Phosdrin^R, an OP insecticide, was able to affect both the ACh receptor and the ion channel associated with it, but without affecting AChE itself. They also found, using two different methods, that physostigmine and neostigmine, in addition to producing blockade of AChE, potentiated the muscle response to ACh when applied in the presence of complete AChE blockade. Albuquerque's studies with OP compounds (46) have suggested an additional effect on the ionic channel that is unrelated to the sites of reaction of ACh on AChE or on the ACh receptor. The simple presence of OP compounds makes the reaction of many agonists, particularly ACh and anatoxin (AnTX), more intensive and speeds the effects of some compounds that block the ion channel. Several agents that react both with the ACh receptor and the channel appear to antagonize the action of the OP compounds in this manner. Conversely, the binding rate of OPs is increased when increasing concentrations of the agonist are present (46), and the rate of binding induced by the presence of large quantities of agonist can occur whether ACh is the agonist or other agents--such as AnTX, subaryldicholine, and succinylcholine--are used in place of ACh.

PRESYNAPTIC ACTION

Anti-ChE agents have presynaptic effects that are related mostly to a reaction with the presynaptic nerve terminal (47-55).

A number of workers reported that compounds such as neostigmine and physostigmine augment and prolong spontaneous release of ACh (miniature endplate potentials or MEPPs) and increase the size of endplate potentials (EPPs) (48,56-59). Boyd and Martin (48) reported a biphasic effect of ChE inhibitors: an increase at low concentrations and a decrease at high concentrations. In a detailed study of the action of neostigmine, ambenonium, edrophonium, and methoxyambenonium on cat tenuissimus muscle, Blaber and Christ (60) reported that MEPP frequency was increased by several ChE inhibitors, and concluded that the effect is probably not related to ChE inhibition but might be related to excitation-secretion coupling, the process by which an action potential releases ACh.

The only published study on presynaptic effects of the more potent ChE inhibitors is that of Abraham and Edery (49), who examined the effect of soman on synaptic transmission in rat diaphragm. In vitro, soman increased the frequency of MEPPs (an effect blocked by Mg^{2+}), caused muscle depolarization that reversed spontaneously, and increased quantal content. It was suggested that the observed changes in transmitter release resulted from an effect on the action potential invading the nerve terminal, although no direct evidence was offered.

In addition, several ChE inhibitors generate antidromic action potentials in motor nerves; these may occur spontaneously (57,61,62) or after an orthodromic nerve volley (47,50,62-64). The potentials are apparently not caused by action potentials originating in muscle, inasmuch as the antidromic repetitive firing has been observed in nerves from muscle incapable of twitching (62). Riker *et al.* (50) and Werner (51,52) concluded that the antidromic discharges were produced by direct actions on the motor nerve terminal. Although ChE inhibition, with later accumulation of ACh and an increase in extracellular potassium concentration, may be the cause of the observed effects, these possibilities are unlikely.

NERVE MEMBRANE

Another example of an effect apparently unrelated to ChE inhibition is found in studies of the actions of ChE inhibitors on ionic conductances of electrically excitable membranes. When single frog nerve fibers were used, physostigmine (1-10 mM) attenuated action potential and current, markedly prolonged the duration of the current, and slowed conduction. This mechanism might be involved in functional sensory deficit and--if selective for inhibitory fibers, as is the case with local anesthetics (65)--might play a role in generation of facilitation before depression.

CENTRAL NERVOUS SYSTEM

The situation is still more complex in the CNS. Even if the action of anti-ChEs were limited to the inhibition of postsynaptic AChE, the complex circuitry of the brain provides ample opportunity for effects at other sites. Because brain cholinergic pathways are diffuse and connect with many other systems, overactivity or blockade of cholinergic synapses can lead to abnormal activity in many other neurons. Apparently, there is no end to the list of transmitters and bioactive substances that can be affected indirectly or directly by cholinergic agonists (66). Among the effects in question are those on the γ -aminobutyric acid (GABA) system which are important in brain excitability and epileptogenesis (67), as well as those involving peptide transmitters and bioactive peptides (68). It is unknown whether these effects are brief or long-lasting. For example, it is unlikely that a perturbation in GABA content would be long-lived after the initial effect of the anti-ChE on the GABA system. However, the circuits are complex, and even a temporary perturbation might lead to reverberations that persist for a long time. The CNS has only a limited capacity to regenerate, and recovery after tissue damage might be slow or incomplete. It is also known that a brief presence of excess transmitter in a synapse can lead to compensatory changes in the number of postsynaptic receptors.

"NEUROTOXIC" ESTERASE (NTE)

Some OPs react with a poorly characterized enzyme--"neurotoxic esterase" (NTE)--of unknown function resident in CNS, PNS, and some other tissues and precipitate a delayed CNS-PNS distal axonal degeneration, which is expressed clinically as a sensorimotor

neuropathy (69). Although a causal association between NTE inhibition and delayed neurotoxicity has not been demonstrated, they are strongly correlated.

NTE is operationally defined as the esteratic activity against phenyl phenylvalerate (the preferred substrate), phenyl valerate, or closely related esters that is "resistant" to paraoxon and sensitive to DFP and mipafox (N,N' -diisopropylphosphorodiamidic fluoride). Two groups of compounds inhibit NTE (Figure 5): one group consists of various phosphates, phosphoramidates, and phosphonates, which induce neuropathy; and the second contains sulfonates, phosphinates, and carbamates, which do not. The latter can react covalently at the phosphorylation site involved in delayed neurotoxicity and thereby protect against later doses of OP compounds that would otherwise induce neuropathy. In contrast, neuropathic OP compounds induce irreversibly (aged) inhibited NTE. Aging involves transformation of the phosphorylated enzyme to a further modified form in which one R group has been cleaved from the phosphorus and a negatively charged residue remains attached to the enzyme. Once this process occurs, regeneration of the active site of the enzyme is no longer possible, and neuropathy will ensue if the NTE-inhibition threshold has been reached or exceeded within a required period. The relationship between this phenomenon and the onset of axonal degeneration is unknown, but it seems likely from recent experimental studies with DFP (70,71) that the target site in nervous tissue is in the nerve fiber itself.

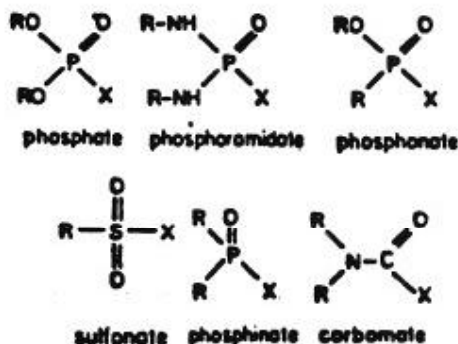


Figure 5. Two groups of NTE inhibitors; Group A (upper) induce delayed neuropathy, Group B (lower) protect against the neuropathic potency of the upper group. R and R' may be alkyl, aryl or heterocyclic substituents; X is the leaving group which is ejected when the inhibitor reacts covalently with the enzyme. Redrawn from Johnson, M.K., 1974: A comparison of the structures of inhibitors of "neurotoxic esterase". J. Neurochemistry 23:786, Fig. 1, Raven Press, N.Y.

BIOCHEMICAL AND METABOLIC CHANGES

OP and related drugs have effects on dehydrogenases and on gluconeogenesis and oxygen uptake (6,72). Failure to gain weight

normally accompanies chronic intoxication with some OP agents, and weight loss accompanies OP-induced neuropathy. Some of these biochemical changes may not be specifically related to the anti-ChEs; e.g., changes in blood lactate and acid-base relationships could be related to severe hypoxia, and the hyperglycemia could result from discharge of catecholamines via cholinergic control of the adrenal medulla.

OP ALKYLATION

Some organophosphates are strong alkylating agents in vitro (73,74). Examples are tetramethyl pyrophosphate, dichlorvos, methyl paraoxon, tetrachlorfenvinphos, mevinphos, crotoxyphos, and methyl parathion. Review of several studies on the alkylation of DNA with dichlorvos indicated that dichlorvos induced methylation in isolated DNA or in DNA of bacterial and animal cells treated in vitro. The extent to which OP esters alkylate DNA in vivo is not clear. On the one hand, the existence in mammals of considerable extrahepatic esterase activity provides an important detoxifying capability that diminishes the chances of deleterious alkylation (74,75). On the other hand, it was reported (76) that [^{14}C]parathion administered in the diet or by intraperitoneal injection resulted in binding of metabolites to liver DNA. It was also assumed that the esterase detoxification pathways may operate only slowly, or not at all, for simple alkyl phosphates like trimethyl phosphate (74,75).

BIOLOGIC AND CLINICAL EFFECTS

ACUTE EFFECTS: PHARMACOLOGY

The characteristic acute pharmacologic effects of the anti-ChE agents classically are ascribed to the inhibition or inactivation of AChE at sites of cholinergic transmission, with the consequent accumulation and action of endogenous ACh liberated both by stimulated cholinergic nerve and (in much smaller amounts) by continual leakage during the resting stage. AChE is present in most tissues in a quantity in excess of that required for normal function; to exert a marked effect in vivo, an anti-ChE agent must generally inhibit 50-90% of the functional AChE at a given site. This can be achieved readily, because most of the anti-ChEs produce 50% inhibition of the enzyme at concentrations of 10^{-7} M or lower.

In principle, it should be possible to predict the pharmacologic properties of anti-ChE agents merely by knowing the loci at which ACh is physiologically released by nerve impulses, and the responses of the corresponding effector organs to the chemical mediator. Potentially, the anti-ChE agents can produce all the following effects: stimulation at autonomic parasympathetic effector organs; stimulation, followed by depression or paralysis, of skeletal muscle and of all autonomic ganglia (nicotinic actions); and stimulation, followed by depression, of cholinceptive sites in the CNS. Because cholinergic stimulation of ganglia increases activity in the sympathetic, as well as the parasympathetic, postganglionic nerves, all the autonomic effectors are activated.

These assumptions are only broadly correct, inasmuch as, with smaller doses of anti-ChEs, particularly those used therapeutically, several modifying factors are present. Most importantly, an

enormous number of compounds can inhibit cholinesterase, and no two are identical in either biochemical properties or pharmacokinetics. Thus, although they all share some general characteristics, the detailed effects vary considerably from compound to compound. For example, compounds containing a quaternary ammonium group do not penetrate cell membranes readily; hence, some anti-ChE agents are excluded by the blood-brain barrier from exerting substantial action on the CNS. But quaternary ammonium compounds act relatively strongly at the neuromuscular junctions of skeletal muscle through both their anti-ChE and their direct cholinomimetic mechanisms and have comparatively less effect at autonomic effector sites. Their ganglionic actions are generally intermediate. The more lipid-soluble agents, such as tertiary amines and most OP compounds, have ubiquitous effects at both PNS and CNS cholinceptive sites.

The main acute pharmacologic actions of anti-ChE agents that are of concern here are those on the eye, the intestine and other organs innervated by the autonomic division of the PNS, the skeletal neuromuscular junction, and the brain. Effects of cholinergic and adrenergic stimulation on effector organs are summarized in Table 1.

Eye

When applied to the conjunctiva, anti-ChE agents cause conjunctival hyperemia and constriction of the iris sphincter (miosis) and ciliary muscle (spasm of accommodation). Miosis is apparent in a few minutes and becomes maximal in 0.5 h. The pupil may be pinpoint sized, but it generally contracts even further when exposed to light. It returns to its normal size in a few hours to several days, depending on the drug and its concentration. The spasm of accommodation is more transient and generally wanes considerably before termination of the miosis. Intraocular pressure usually decreases concomitantly, but in some cases anti-ChE agents may cause an initial increase in intraocular pressure owing to dilatation of the finer blood vessels and increased permeability of the blood-aqueous humor barrier; this is generally followed by a decrease to below initial pressure.

Systemically administered anti-ChEs similarly affect the cholinergic terminals supplying the circular muscles of the eye to produce miosis, but they also affect sympathetic ganglia that operate the apposing radial muscles, and they act on the brain in ways that may reduce activity in cholinergic nerves to the eye. Thus, the effects of systemic administration are not as predictable as those of local administration, and either constriction or dilatation of the pupils may be seen.

Gastrointestinal Tract

Although the actions of various anti-ChE agents on the gastrointestinal tract are nearly identical, neostigmine has been studied most extensively in this regard. In man, neostigmine increases gastric contractions and increases the secretion of acidic gastric juice. The drug tends to counteract the inhibition of gastric tone and motility induced by atropine and increases the stimulatory effect of morphine.

Neostigmine augments the motor activity of the small and large bowel; the colon is particularly stimulated. Atony is overcome or prevented, propulsive waves are increased in amplitude and frequency, and transport is thus promoted. Atropine inhibits, but

does not abolish, the intestinal effects of neostigmine. The total effect of anti-ChE agents on intestinal motility probably represents a combination of actions at the ganglion cells of Auerbach's plexus and at the muscle fibers, as a result of the preservation of ACh released by the cholinergic preganglionic and postganglionic fibers, respectively.

Actions at Other Autonomic Sites

Secretory glands that are innervated by postganglionic cholinergic fibers include the bronchial, lacrimal, sweat, salivary, gastric, intestinal, and acinar pancreatic glands; low doses of anti-ChE agents cause, in general, an augmentation of their secretory responses to nerve stimulation, and higher doses increase the resting rate of secretion.

Smooth muscle fibers of the bronchioles and ureters are contracted by these drugs, and the ureters may show increased peristaltic activity.

The cardiovascular actions of anti-ChE agents are extremely complex, in that they reflect at any given moment the sum of the excitatory and inhibitory actions of accumulated endogenous ACh at several levels. The predominant cardiac effect of the peripheral action of accumulated ACh is bradycardia, which results in a decrease in cardiac output and in hypotension. The effective refractory period of cardiac muscle fibers is shortened, and the refractory period and conduction time of the conducting tissue are prolonged. The blood vessels are in general dilated, although the coronary and pulmonary circulation may show the opposite response. The sum of the foregoing effects should result in hypotension, but at the ganglionic level ACh has first an excitatory and, at higher concentrations, an inhibitory action. Hence, the excitatory action on parasympathetic ganglion cells tends to reinforce the above effects, whereas the opposite sequence results from the action of ACh on sympathetic ganglion cells. Excitation followed by inhibition is also produced by ACh at the medullary vasomotor and cardiac centers. All these effects are further complicated by the hypoxia resulting from bronchoconstriction and other actions on the respiratory system. The hypoxia reinforces both sympathetic tone and ACh-induced discharge of epinephrine from the adrenal medulla. It is not surprising, therefore, that a wide variety of hemodynamic effects of anti-ChE agents has been reported, depending on drug, dose, route of administration, species, and other factors.

Neuromuscular Junction

The actions of anti-ChEs are thought to be due to inhibition of ChE at the motor endplate and retention of ACh at the junctional region. This combination culminates in a marked reaction of the transmitter with the receptor and a great activation of the entire receptor-ion-channel complex. If this process continues, a number of undesirable reactions can occur, among them paralysis, the desensitization of the junctional receptor, and structural changes in the muscle and nerve ending.

Normally, a single nerve impulse in a terminal motor axon liberates enough ACh to produce a localized depolarization (the endplate potential) that initiates a propagated muscle action potential. The liberated ACh is rapidly hydrolyzed by AChE, and the muscle relaxes. Therefore, each motor-nerve impulse initiates only one muscle contraction. After partial inhibition of AChE, however,

the ACh liberated by a single nerve impulse may persist long enough to set up repetitive muscle action potentials, with a resulting increase in strength of contraction. Furthermore, sufficient ACh may diffuse to neighboring muscle fibers and excite them as well, causing asynchronous contractions (fibrillation). In addition, the action of anti-ChE agents on the axon terminal can initiate antidromic firing, which results in activation of the motoneuron and leads in turn to the synchronous contraction of an entire motor unit (fasciculation). In the presence of a sufficiently high dose of an anti-ChE agent, the local concentration of ACh may produce a depolarizing blockade of the neuromuscular junction and paralysis. Thus, a small dose of anti-ChE may increase the skeletal muscle contraction produced by a single maximal nerve stimulus, but larger doses, or repetitive nerve stimulation at a high physiologic rate, may result in depression or block of neuromuscular transmission.

Brain

The mechanisms by which anti-ChEs perturb brain function are more complex, harder to study, and consequently less well understood. The brain is an extraordinarily complex network of neurocellular pathways which uses electrochemical mechanisms to conduct signals needed to perform and integrate cognition, awareness, memory, language, sleep and wakefulness, locomotion, sensation, and hormonal and autonomic functions. Cholinergic neurons are probably involved in many intraregional and interregional pathways of the brain, although their identity and specific functions are poorly understood. Several sets of presumptive central cholinergic pathways have been proposed: medial septal nucleus to dentate gyrus, and subiculum of hippocampus habenula to interpeduncular nucleus; cortical internurons to cortical pyramidal neurons; and thalamus, putamen, and caudate to neurons in the caudate (77). The presence of cholinergic synapses in central motor pathways (pyramidal and extrapyramidal) and of afferent systems involving both the reticular formation and the thalamus, hippocampus, and limbic system, suggests the possibility of their participation (with other types of chemical synapses) in initiation and control of movement, in sleep, arousal, and wakefulness, in memory, and in emotional regulation. This, in turn, implies susceptibility of these functions to anti-ChEs and cholinomimetic drugs.

The electroencephalogram (EEG), a record of changes in the voltage-field distribution over the head as a function of time, is a tool for studying some aspects of brain function. Adults have characteristic EEG patterns under standard conditions, but these vary according to the state of consciousness (alert, startled, drowsy, dreaming, or deeply sleeping). Awake subjects display a high-voltage (50 μ V), low-frequency (8-14 Hz) alpha rhythm. This is most prominent in the occipital region of the scalp and when the eyes are closed. The resting alpha rhythm is replaced during periods of attention and problem-solving by a low-voltage (5-10 μ V), high-frequency (15-30 Hz) beta rhythm, most prominent in frontal and parietal regions. The changeover from alpha to beta rhythm, termed "desynchronization", can be induced by mental concentration or external stimuli (including anti-ChEs). Other frequencies of electric activity commonly observed in the EEG are theta (4-7 Hz) and delta ($\leq$ 3 Hz) waves.

Among brain functions, sleep is particularly well studied with the EEG. When sleep occurs, the alpha pattern disappears and, over a period of 4-5 min, the EEG changes from a low-voltage to a higher-amplitude, 4- to 6-Hz pattern with intermittent 14- to 16-Hz "spindle" activity. Later, over the course of 1-2 h, the voltage increases, the frequency decreases (to 1-3 Hz), and spindle activity becomes less frequent. The EEG then becomes desynchronized, rapid eye movement (REM) occurs, and the person dreams. REM sleep lasts approximately 15-20 min and occurs three to five times during a normal sleep cycle of 7-8 h. These characteristics are constant from night to night if the person is healthy, is well adapted to the environment, and has an habitual, normal, 24-h sleep-wake cycle.

The control of the sleep-wake cycle appears to be a complex phenomenon involving several groups of neurons (nuclei) in the brainstem that use different synaptic transmitter chemicals: the locus ceruleus (norepinephrine), the dorsal raphe nuclei (serotonin), and the gigantocellularis nuclei (ACh) of the pontine reticular formation (78). Cholinergic neurons in the gigantocellularis nuclei concentrate their electric discharges during REM sleep: activity begins minutes before the onset of REM sleep, continues at a high rate during the REM period, and abruptly ceases as REM sleep terminates. An attractive postulate of the control of the sleep-wake cycle is that, during non-REM sleep (and waking), an inhibitory system composed of neurons in the locus ceruleus or the dorsal raphe nuclei tonically prevents cholinergic neurons in the gigantocellularis nuclei from firing. At a critical point, the latter escape this inhibition and fire at very high rates, thereby initiating the REM period. The inhibitory neurons then increase their activity and inhibit the cholinergic discharge, and REM sleep ceases. Dependence on ACh as the excitatory transmitter implies the sensitivity of this system to anti-ChEs, which, a priori, tend to increase neuronal activity. This postulate is consistent with empirical evidence from humans and animals treated with cholinergic agonists, which tend to decrease the latency of REM sleep and increase the number of REM-sleep episodes. Atropine exerts opposite effects.

Table 2 lists some of the effects of anti-ChEs and cholinomimetic drugs on brain function (79-84). Doses of OP compounds that are toxic, but too small to threaten life, produce a variety of clinical manifestations, including miosis, muscular fasciculation, and apprehension (85,86). The acute behavioral alterations are usually accompanied by marked desynchronization of the EEG (87). Larger doses of OP compounds--which may induce convulsions, muscular paralysis, and death--cause slowing of the EEG pattern followed by the appearance of spike waves that herald the onset of seizures. Symptomatic recovery is normally complete within 2-9 wk, at which time the erythrocyte cholinesterase content usually has returned to normal (88,89).

Repeated low-dose administration of OP compounds can produce symptoms and signs that are not seen after single exposures to the same doses. For example, subjects given daily injections of DFP reported the additional symptoms of insomnia, excessive dreaming, emotional lability, increased libido, paresthesias, visual hallucinations, and tremor (90); and prolonged administration in animals induces sensorimotor neuropathy.

The subjective manifestations of brain dysfunction usually disappear shortly after cessation of exposure; in most instances, EEG abnormalities reportedly disappear within 2 wk of acute exposure or termination of chronic exposure (90-92).

ACUTE TOXIC EFFECTS

Toxicity of particular OP compounds does not vary greatly among mammals. Signs and symptoms differ mainly in sequence and in individual prominence (93-96). Rapidity of appearance, intensity, and timecourse depend on dose and route of administration.

The effects of acute intoxication with anti-ChE agents are manifest by muscarinic and nicotinic signs and symptoms and, except for compounds of extremely low solubility in lipids, signs referable to the CNS. Effects may be local or general. Local effects are due to the action of vapors or aerosols at their site of contact with the eyes or respiratory tract or to the local absorption after liquid contamination of the skin or mucous membranes, including those of the gastrointestinal tract. General effects rapidly follow systemic absorption by any route; they appear most rapidly after inhalation of vapors or aerosols, in which case severe effects may appear within a few minutes. In contrast, the onset of symptoms after gastrointestinal and percutaneous absorption is delayed. The duration of effects is determined largely by the nature of the compound; it may vary from minutes, as after an overdose of edrophonium, to several days or even weeks after irreversible alkylphosphorylation of AChE, as by DFP or sarin.

After exposure to vapors or aerosols or after inhalation, ocular and respiratory effects generally appear first. Ocular effects include marked miosis, conjunctival congestion, ciliary spasm, and browache, along with watery nasal discharge; respiratory effects consist of "tightness" in the chest and wheezing due to the combination of bronchoconstriction and increased bronchial secretion. After ingestion, gastrointestinal effects appear first, including anorexia, nausea and vomiting, abdominal cramps, and diarrhea. After percutaneous absorption of liquid, localized sweating and muscular fasciculation in the immediate vicinity are generally the earliest manifestations. Severe intoxication is manifest by extreme salivation, involuntary defecation and urination, sweating, lacrimation, bradycardia, and hypotension.

Nicotinic actions at the neuromuscular junctions of skeletal muscle usually consist of fatigability and generalized weakness, involuntary twitching, scattered fasciculation, and eventually severe weakness and paralysis. The most serious consequence of the neuromuscular actions is paralysis of the respiratory muscles.

The effects on the CNS include confusion, ataxia, slurred speech, loss of reflexes, coma, and central respiratory paralysis. Actions on the vasomotor and other cardiovascular centers add to the peripheral actions to complicate the hemodynamic pattern. After large doses or inhalation of high concentrations, the time course may be telescoped into a few minutes and many of the above signs overshadowed by dyspnea, apnea, and collapse. A case of severe accidental poisoning in man is illustrative (97). Development of signs after smaller doses has been described by Grob (98,99) and others (100-104).

After a single exposure, death may come within 5 min or not for some 24 h, depending on dose, route of administration, drug, and other factors. The cause of death is primarily respiratory failure, usually accompanied by a cardiovascular component. Muscarinic, nicotinic, and central effects all contribute to respiratory embarrassment; they include laryngospasm, bronchoconstriction, increased tracheobronchial and salivary secretion, and peripheral and central respiratory paralysis. Although the blood pressure may fall alarmingly and cardiac irregularities may intervene, these effects probably result as much from hypoxia as from the specific actions mentioned, inasmuch as they can often be reversed by the establishment of adequate pulmonary ventilation.

Differences in hazard among members of the OP group arise from differences in inherent potency (EA 3148 is the most potent), vapor pressure (GB is hazardous by inhalation), and ability to penetrate the skin (VX and GD) (102,105,106). GB as a liquid administered cutaneously has also been reported to cause severe poisoning (107, 108). Absorption of some of these compounds from the respiratory tract has been estimated to approach absorption after intravenous administration in completeness (105,109,110). Incapacitating concentrations of GB and others by inhalation have been estimated in animal tests, (111) and extrapolations to man have been attempted (105,112,113).

Two antidotes are currently used for acute poisoning by anti-ChEs. One is atropine, which acts as a muscarinic receptor antagonist and reduces the excessive stimulation of parasympathetic functions, thus reversing the effects on the eye, lung, gastrointestinal muscles, and, most important, the heart. Atropine also relieves effects of poisoning at central muscarinic synapses, but not at central nicotinic synapses. The other antidote is an oxime (such as 2-PAM or obidoxime) that relieves poisoning at skeletal muscle endplates (20). It acts as an activator of phosphorylated AChE; through nucleophilic attack, it removes the phosphate group, thus restoring enzyme function at motor endplates. The oximes are inefficient if the phosphorylated enzyme has aged. These oximes contain quaternary nitrogen and therefore do not penetrate the blood-brain barrier and have no effect at cholinergic synapses in the brain or spinal cord.

The acute effects of the anti-ChEs are short-lived and do not outlast the inhibition of the enzyme. Indeed, some systems develop tolerance rapidly, so function returns to normal even before there is substantial regeneration of measurable enzyme activity. It has been amply documented that, even with over 99% inhibition of all ChEs, animals (and presumably humans) can survive without oxime or atropine treatment, if they are supported for a couple of hours by pharmacologic or nonpharmacologic means, such as artificial respiration, (72). This recovery from the "irreversible" effects of inhibitors may depend on rapid regeneration of ChEs, particularly some AChE isoenzymes (114); desensitization of the postsynaptic membrane, a phenomenon that limits the response to accumulated ACh; or compensatory changes in presynaptic and postsynaptic receptors (115).

DELAYED NEUROPATHY (CNS-PNS DISTAL AXONOPATHY)

Clinical Features

Degeneration of particular regions of the nervous system is a well-characterized adverse health effect of human and animal exposure to many OP esters (phosphates, phosphoramidates, and phosphonates) that may or may not also display anti-AChE properties. Some neuropathic OP esters can precipitate prominent neurologic abnormalities after a single exposure (as well as after multiple exposures), the clinical disease usually beginning within 2-3 wk. At some time during this clinically quiescent period, a stereotyped sequence of neuropathologic changes takes place that leads to the appearance of sensorimotor neuropathy. The degree of clinical impairment and the prognosis for functional recovery depend directly on the extent of nervous system damage, which in turn depends on the neuropathic potency of the responsible OP compound, as well as the dose and duration of exposure.

The first recorded cases of paralysis from OP intoxication occurred at the end of the nineteenth century, when patients with tuberculosis were treated with phosphocresote, an uncharacterized mixture of esters derived from phosphoric acid and coal-tar phenols (116). Several thousand cases appeared in the southern states in 1930 when alcoholic extracts of Jamaica ginger, widely consumed during Prohibition, were adulterated (117) with 2% triorthocresylphosphate (TOCP). Adulteration of cooking oil contaminated with lubricating oil containing cresyl phosphates has proved responsible for several outbreaks of OP neurotoxicity, including a major epidemic in Morocco in which more than 10,000 people reportedly were affected (118). More recently, the OP pesticide leptophos has been associated with an outbreak of occupational neurotoxicity among workers at a plant in Texas; the victims displayed pronounced clinical features of spinal-cord damage, and some had psychologic manifestations (119).

Approximately 2 wk after ingesting cresyl phosphates, during which gastrointestinal disturbances may be manifest, affected persons experience pain, aches, and tingling in the feet and calves, followed within days by progressive weakening of leg and foot muscles that leads to paralysis. The thighs and then the hands and arms may become weak during succeeding days. Weakness is always more severe in the legs than in the arms and in both limbs is greatest in distal muscles. During the progressive phase of the illness, which lasts 1-2 wk (depending on dose), the weakness spreads steadily, but usually stops short of complete quadriplegia. Neurologic examination reveals signs of damage to the spinal cord (hyperactive knee jerks) and peripheral nerves (hypoactive ankle jerks). Foot drop is pronounced, and victims adopt a high-stepping gait. Muscle denervation is evident from electromyography, and atrophy in the lower legs and hands may become severe. Recovery in mild cases takes months or years, but severely affected persons who recover some muscle strength may have ataxia and spasticity permanently (120).

Many experimental species are vulnerable to the delayed neurotoxic effects of OP compounds, such as TOCP, although it is accepted that neurotoxic doses vary markedly from one species to another. Rodents are relatively resistant and fowl very susceptible; hens are widely used to assay AChE compounds for

ability to induce paralysis (121). In all sensitive species, there is a period of 1-2 wk before the onset of neurologic signs, during which body weight changes and nerve fibers degenerate. Hens given TOCP develop a steadily increasing flaccid paresis of the hindlimbs, with an ataxic, broad-based gait. Paresis spreads over the course of several days and, if respiratory muscles become involved, may lead to death.

Neuropathologic Features

The limited neuropathologic information available from studies of affected persons demonstrates that OP poisoning induces degeneration of nerve fibers in spinal cord and peripheral nerves (122).

Neuropathologic examination of experimentally poisoned animals reveals a characteristic pattern of distal, retrograde degeneration of peripheral nerves. Long nerve fibers of large diameter seem to be affected before shorter and smaller fibers, so sensory and motor manifestations of nerve damage generally affect the legs before the arms. A similar principle holds for involvement of the spinal cord: long ascending (gracile and spinocerebellar) and long descending (e.g., corticospinal) tracts are symmetrically involved in the degeneration process. Neuropathologic changes initially appear distally and multifocally in affected pathways, leading to degeneration of distal axons and structural and functional disconnection of sensory and motor terminals. Axonal degeneration progresses steadily toward, but stops short of, the nerve cell bodies (71). Loss of axons precipitates a secondary loss of the normal myelin sheath in affected regions of spinal cord and peripheral nerves; this phenomenon has been erroneously described as "demyelination"—a term reserved to describe the neurotoxic properties of substances, such as hexachlorophene, that damage myelin without causing axonal degeneration. In sum, the clinical and pathologic features of delayed OP neuropathy are classified as a central-peripheral distal axonopathy (123).

Neuropathic Potency

Neuropathic potency can be assayed by determining the response of a vulnerable species (the hen, Gallus gallus domesticus) to OP intoxication or predicted from the degree of inhibition of the nervous-system enzyme NTE. It is important to note that chemical reactivity of an OP compound with NTE is unrelated to its ability to inhibit AChE (69). Some OP agents designed for chemical warfare can inhibit both NTE and AChE. However, doses needed to inhibit NTE and induce neuropathy may be much higher than those which would prove fatal to animals or humans without protection against the acute anti-ChE effects. Alternatively, the degree of NTE inhibition may be insufficient to induce clinical neuropathy. Phosphorofluoridates induced delayed neuropathy in chickens after 9-15 d at doses of 0.3-2.5 mg/kg; the dimethyl compound required doses of 30 mg/kg. Five alkylphosphorofluoridates were active at 1-5 mg/kg given in divided daily doses. Diethyl phosphorofluoridothionate induced neuropathy at 0.75 mg/kg, but not at 0.5 mg/kg. Various dialkylphosphinic fluorides and dialkylpyrophosphonates were negative (124). Neuropathologic studies designed to detect subclinical damage to spinal cord or peripheral nerves in animals treated with CW agents are not available.

LONG-TERM BRAIN DYSFUNCTION

Several studies have suggested that some subjects experience long-term sequelae from a single OP exposure or a period of low-level exposure. Minor disorders of affect, emotion, and memory were reported by Tabershaw and Cooper (125) in 38% of 114 subjects after acute OP poisoning. Rowntree *et al.* (126) suggested that OP exposure might exacerbate psychiatric problems. Metcalfe and Holmes (82) claimed that OP exposure may lead to persistent EEG changes; they also reported that workers with histories of both OP and chlorinated-hydrocarbon exposure, but with no recent exposures, had EEG patterns that showed excessive slowing during drowsiness and after hyperventilation. Moreover, all-night-sleep EEGs reportedly displayed patterns commonly associated with narcolepsy. Psychologic dysfunction in this group included disturbed memory and difficulty in maintaining alertness and appropriate focusing of attention.

Duffy and co-workers (127) examined the brain electrical activity of workers occupationally exposed to sarin and with documented single or repeated accidental exposure to toxic concentrations of it at least a year before EEG recording. Standard clinical EEG measurement, computer-derived EEG spectral analysis, and standard overnight-sleep EEGs, were examined in 77 exposed workers, and the results were compared with those from a control group of 38 nonexposed industrial workers. Statistically significant group differences in sarin workers included increased beta activity, delta and theta slowing, decreased alpha activity, and increased REM sleep.

The results of Duffy *et al.* were consistent with those from a previous study that examined EEG changes in rhesus monkeys exposed to sarin or diethrin (87). Two dose schedules were used: a single "large dose" (sarin at 5 µg/kg or diethrin at 4 mg/kg, administered intravenously), which produced overt signs of toxicity, and a series of 10 weekly "small doses" (sarin at 1 µg/kg or diethrin at 1 mg/kg, administered intramuscularly). The effects of anoxia in the first group were precluded by pretreating animals with gallamine triethiodide and providing artificial respiration. Animals treated with single doses of either sarin or diethrin displayed significant increases in the relative amount of beta voltage (15-50 Hz) in the EEG that persisted for a year. For sarin, the predominant effect was in the EEG derivation from the temporal cortex, and for diethrin, from the frontal cortex. For both drugs, the increase in beta activity was most prominent when subjects were awake in darkness or drowsy.

In summary, research results have indicated that a single symptomatic exposure or a series of subclinical exposures to sarin can alter the frequency spectrum of the spontaneous EEG for up to a year (83).

The effect of anti-ChEs on the structural integrity of brain tissue has rarely been investigated, although the destructive effects of some of these compounds on spinal cord and peripheral nerves are well known. There is a clear need to study, with current ultrastructural techniques, the possibility of selective brain damage underlying the reported long-term changes in EEG patterns of animals and humans exposed to anti-ChEs, especially in light of a recent provocative report of widespread axonal and terminal degeneration in the brains of rats treated with soman. As an incidental finding in an unrelated study of the effects of this

agent on behavior of rats, Petras (128) described nerve-terminal degeneration in the limbic system, corticofugal system, and central motor system-areas associated with mood, affect, judgment, emotion, posture, and locomotion. The author pointed out that damaged regions would be unlikely to regenerate and that long-term psychiatric and motor deficits might be anticipated. Only 16 animals were studied by Petras, and only seven brains had observable damage. The seven had significant acute toxicity at the time of exposure, including muscle fasciculations, tremors, and seizures. Only an abstract of this work has been published, and the research has not been pursued systematically. It cannot now be concluded that the tissue damage was a direct effect of the OP, rather than an indirect effect (e.g., related to brain hypoxia). Nor can it be ascertained whether the response was specific for soman.

JUNCTIONAL NEUROMYOPATHY

Pathologic changes develop subacutely and reversibly in some motor-nerve terminals, neuromuscular junctions, and associated muscle fibers after administration of anti-ChE drugs to laboratory animals. ChE inhibitors with long-lasting effects-such as paraoxon, DFP, tabun, sarin, soman, and parathion-and "reversible" inhibitors, such as physostigmine and neostigmine, induce these neuromyopathic changes (129-131). The effect may result not only from AChE inactivation, but also from an increase in the rate of spontaneously released ACh secondary to a prejunctonal action of anti-ChE drugs (54,132). Thus, guanidine, which increases ACh release, also causes a subacute myopathy similar to that produced by anti-ChE agents (133).

The diaphragm is most severely affected in treated animals (134), but abnormalities are also prominent in the soleus, gastrocnemius, and quadriceps muscles. Ultrastructural changes may appear within hours of drug administration, progress over a period of a few days, and resolve within a couple of weeks. Pathologic changes in motor-nerve terminals, neuromuscular junctions, and muscle fibers are associated with an initial decrease in contractile strength after a few days and then a return to nearly normal strength after several more days (135). These pathophysiologic events may be clearly delineated from the degeneration of motor-nerve terminals and atrophy of muscles that follow administration of agents that induce delayed neuropathy (131), in that subacute neuromyopathic changes not only resolve before the onset of delayed retrograde axonal degeneration, but also fail to develop with TOCP, an O-P compound that inhibits NTE but has little AChE activity. They may also be prevented by protecting the neuromuscular junction with curare or a reactivator of phosphorylated ChE, such as 2-PAM. The myopathic process seems to depend on the degree and duration of ChE inhibition (136); this suggests that skeletal-muscle hyperactivity is causally associated with the phenomenon.

The significance of these observations for humans exposed to OP agents is unknown, but it seems likely from animal experiments that myopathy does not develop in the absence of muscle hyperactivity induced by anti-ChEs. There is some evidence from human autopsy material of focal necrosis of diaphragmatic and intercostal muscles after accidental exposure to a single large dose of OP insecticide (137).

Motor-nerve terminals show various degrees of subcellular changes within 30 min to 2 h after injection of soman or paraoxon (138); soman induces the more severe changes. Nerve terminal alterations include the appearance of intra-axonal myelin figures, membrane enclosures, and an increased number of large-coated vesicles. Three days after DFP injection, soleus motor-nerve terminals are reduced in number and naked endplates are common. Pathologic changes also appear in the subneural apparatus and in the immediate subjacent muscle; the latter displays swollen mitochondria, myelin figures, enlarged nucleoli, dilatation of the sarcoplasmic reticulum, loss of myofibrillar striation, and, later, myofilament loss and fragmentation of Z bands (132). Focal muscle necrosis then ensues. Prejunctional and postjunctional subacute changes are resolved within 2 wk after administration of DFP; however, because this compound also inhibits NTE and induces delayed neuropathy, a week after recovery from the subacute neuromyopathic changes motor-nerve terminals undergo a second phase of degeneration and regeneration, with reinnervation of damaged endplates 6-8 wk later (131).

OTHER ADVERSE HEALTH EFFECTS

Mutagenic or carcinogenic action is not a general feature of the OP compounds, although some may have these properties. There appears to be a lack of information on the mutagenicity or carcinogenicity of carbamate anti-ChEs.

Some agents on the list of compounds under scrutiny belong to chemical classes other than OP compounds and carbamates. Edrophonium is a quaternary ammonium inhibitor of ChE. Methacholine is a stable choline ester and, although urecholine is a carbamate, it is not hydrolyzed by ChE. These two compounds are direct-acting cholinomimetic agents. They are approved for use in clinical medicine, but have few indications. Hexafluorenum is a bisquaternary ammonium distantly related to tubocurarine, and it has both anti-ChE and curariform actions. None of these quaternary ammonium compounds is known to have genotoxic action. Another compound is tacrine, 9-amino-1,2,3,4-tetrahydroacridine; as a class, acridines are notorious for mutagenicity and carcinogenicity, but this particular chemical has been used in clinical practice for more than 20 yr without known sequelae.

Mutagenicity

The OP malathion has been investigated extensively in a number of mutagenesis test systems. Some studies were done with a metabolic activating system, some without (malathion requires metabolic activation for its AChE-inhibiting effect). Malathion also has been tested in somatic cells; tests for chromosomal aberrations in human hematopoietic cell lines, sister chromatid exchange (SCE) in human fetal fibroblasts, and the micronucleus test in mice have been performed. A mouse dominant-lethal mutation assay has also been carried out with malathion, although the study was compromised, in that the dose (300 mg/kg) was well below that which is maximally tolerated. Among these studies, only one (reported in an abstract) claimed positive findings (139). These occurred in the mouse micronucleus and SCE tests and in a host-mediated Salmonella

assay. The findings concerning the mutagenicity of malathion are therefore equivocal.

A 1975 review (140) concluded that the OPs bidrin, dichlorvos, dimethoate, methyl parathion, and oxydemeton methyl, were mutagens in a single microbial mutation assay. In addition, trimethyl phosphate is a clear-cut mutagen in male mouse germ cells; six other OP compounds (bromophos, diazinon, fenitrothion, malathion, parathion, trichlorphon) apparently are not. Results of assays in four microbial test systems were inconclusive for several OPs (141). Further study of these compounds has not been undertaken.

Carcinogenicity

Malathion given in the diet for 103 wk was not carcinogenic in male and female rats, although the dose used may have been less than that which is maximally tolerated. No increased tumor incidence could be found in mice given malathion for 80 wk.

An International Agency for Research in Cancer (IARC) monograph reported strong evidence of the carcinogenicity of trimethyl phosphate (142) in one species and evidence of carcinogenicity in another. There is equivocal evidence of the carcinogenicity of parathion and dichlorvos in animals. A single dose of dichlorvos reportedly damages the germinal epithelium of mouse testes (143).

With the exception of urethane (ethyl carbamate), a potent mutagen and established carcinogen in laboratory animals (144,145), carbamate compounds have been largely unstudied.

Fetal and Teratogenic Effects

Parathion reportedly increases resorption and lowers the weight of rat fetuses (146). Results for malathion are questionable (147). No data appear to be available on the potential mammalian teratogenicity of military nerve agents.

Effects on Male Reproduction

A single dose of dichlorvos reportedly damages the germinal epithelium of mouse testes severely (143).

Cataractogenic Effects

The introduction of DFP and other long-acting anti-ChE agents in the late 1940s appeared to represent an important advance in the treatment of open-angle glaucoma. These drugs maintained control of intraocular tension when administered frequently and were effective in cases that were no longer controllable with the shorter-acting compounds. Then, in 1966, independent reports simultaneously implicated the long-acting anti-ChE agents in the causation of lenticular opacities (148, 149). The incidence of this effect appeared to be as high as 50%; its development varied directly with the strength of the solution, frequency of instillation, duration of therapy, and age of the patient. With physostigmine and other short-acting anti-ChE agents, the occurrence of lenticular opacities appeared to be no greater than that in nontreated subjects in the same age groups.

The mechanism of the cataractogenic action of the long-acting anti-ChE agents has remained obscure (150). It has been shown that vervet monkeys can serve as a model for the production of an identical picture with the daily, long-term instillation of echothiophate (151). In an extensive series in which children were treated for accommodative esotropia (strabismus) with the instillation of short- and long-acting anti-ChE agents, no lenticular opacities or other serious effects were detected (152).

Hemocoagulation

Aberrations of the clotting mechanism attend exposure to OP anti-ChEs (153). Study of 31 persons exposed to sarin or parathion revealed a biphasic reaction consisting of rapid coagulation immediately after exposure, followed by a prolongation of clotting time (91,154). Anti-ChEs also increase fibrinolysis. Fibrinolysis and hypercoagulability were noted in two patients with mevinphos poisoning (155). Increased coagulation was associated with increased prothrombin activity (secondary to increased Factor VII activity) and with increased prothrombin consumption. Coagulation abnormalities normalize several weeks after exposure, and there is no overt liver damage.

IMMEDIATE AND DELAYED EFFECTS IN VOLUNTEERS AFTER SINGLE OR REPEATED EXPOSURE TO ANTI-CHES AT EDGEWOOD

According to the available information, 1,406 subjects were tested with 16 agents of unstated purity (Table 3-3). Some of the subjects also were treated with protective or reactivating agents (case file data of Edgewood subjects, Appendix E).

For this review, approximately 15% (219) of the medical records were selected on the basis of high dosage, repetitive exposure, or the presence of additional physiologic stress. Case records were also selected randomly on the terminal digit of the case number (i.e., ending in 3). Brief summaries were made available to the Panel. In addition, complete records of 32 persons given EA 3148 intravenously were examined. These were chosen because this agent is considered the most potent of the anti-ChEs tested (156). In all cases, subjects were identified by pharmacologic class, by agent, and by letter code; names were withheld.

The case summaries are brief and anecdotal. With the exception of one case, they deal only with the period immediately after the test dose. There are no reports of neurologic or psychologic examinations (with the exception of subject A5J), and only four reports of EEGs—three made before treatment with the anti-ChE agent, and one after. The medical records of subjects tested with EA 3148 comprise doctors' orders, observations of the patient recorded largely by nurses, a clinical master log recording only blood pressure and pulse before and after exposure, results of a test of numerical facility, and ChE concentrations in blood, plasma, and red cells, before and at intervals after exposure. Physician's workup, progress, and discharge notes are absent. Descriptions of the subjects' reactions are rather vague and are certainly not sufficient for careful analysis of long-term effects of these agents. The various subjective complaints listed in the case-file data of Appendix E are not further documented by examination of findings.

The major focus of this Panel's investigation is the possibility of long-term or delayed effects. Essentially, anecdotal information is provided in the case summaries and some of this information seems to suggest that immediate psychological effects can follow the administration of both reversible and irreversible cholinesterases. However, the summaries do not provide hard data that would allow the panel to address, in a definitive manner, the question of whether or not there is a possibility of long-term or delayed effect. As noted above, the case summaries were concerned with the period immediately after administration of the agent and therefore give no indication of the possibility of long-term adverse effects. The paucity of data in the medical records prevents further study in relation to the goal of this report. However, there are published papers which appear to demonstrate long lasting EEG changes from single or repeated exposure to OPs (83).

In reviewing the detailed case report of the single subject who experienced long-lasting psychologic symptoms, the Panel noted that he had had both physical and psychologic evaluations before acceptance in the volunteer program. Evidently, this included examinations and psychologic testing (the MMPI was mentioned). It is possible that these data, if available, can be used as a basis for comparison with a long-term followup study, if such is undertaken.

MORTALITY DATA

A preliminary review of the standardized mortality ratios (SMRs) suggests that mortality was not significantly increased by exposure to anti-ChE chemicals, as tested at Edgewood (Table 4). Whether one looks at subjects exposed to sarin only or sarin plus another chemical (sarin total), VX only or VX plus another chemical (VX total), anti-ChE only or anti-ChE plus another chemical, the SMRs are roughly 80% (or less) of the rates expected for the U.S. population as a whole. This presumably reflects the fact that those who enter the military service do not have chronic diseases. These findings provide a first approximation of mortality and were intended to reveal trends. A more thorough evaluation of mortality findings is contained in Chapter 4.

EVALUATION OF THE LIKELIHOOD OF LONG-TERM ADVERSE HEALTH EFFECTS

The following commentary is based on evaluation of the known adverse health effects of anti-ChEs on humans and animals, the type, number, amount, and route of administration of agents, and medical records of subjects released by the Army.

The subjects were experimentally given anti-ChE agents in the CW testing program during the 1950s and 1960s. The mortality data, collected during 1981, led to the conclusion that, during the elapsed time since testing, subjects were no more likely to have died than comparable soldiers outside the testing program. Morbidity data are unavailable and should be collected; special focus should be placed on the possible long-term adverse health effects highlighted below.

It must be emphasized that the following opinions represent essentially extrapolations from known data. Although there are

published and nonpublished results indicating that EEG changes can occur for one year after exposure to organophosphorus drugs, there is no documentation available as to longer-lasting effects. The statements offered below therefore represent conjecture on the part of Panel members and their consultants, who collectively have broad experience with and expertise on anti-ChEs and their effects in humans and animals.

BRAIN DYSFUNCTION

There are no records to indicate that the soldiers might have experienced subtle changes in brain function that lasted for long periods after discharge from the test environment (except, perhaps, in the one case cited above). But examination of primates and humans exposed to single or repeated doses of sarin has revealed statistically significant changes in the EEG that are apparent for at least a year after exposure (83). These observations have yet to receive laboratory confirmation, and their exact importance is unknown. There are also a number of anecdotal reports of minor disorders of affect, sensation, memory, and sleep in humans accidentally exposed to OP war gases or insecticides. The underlying changes in brain structure and function are unknown. The EEG changes are compatible with abnormalities of sleep and behavior. It is also possible that pre-existing psychopathologic conditions were exacerbated. Some subjects were treated with soman, which has been alleged (in a single, limited experimental study) to induce in laboratory rats profound and irreversible neuropathologic changes in vitally important regions of the brain (128). This laboratory study has not been replicated, and the duration and lifetime significance, if any, of the above-noted effects in humans are not known; they should be looked for in both adult life and old age. There is no evidence from the medical records that subjects given potent anti-ChE chemicals (e.g., military nerve agents) experienced the more severe acute effects that were produced in animals with large doses of soman. In particular, there are no reports of respiratory insufficiency or convulsions that could precipitate periods of hypoxia and lead to permanent damage of brain tissue.

JUNCTIONAL NEUROMYOPATHY AND DELAYED NEUROPATHY

Medical records of the vast majority of subjects tested do not refer to induction of the muscle hyperactivity that is associated with acute neuromyopathy in laboratory animals and humans poisoned by OP insecticides. Although there was no systematic attempt to inspect tested subjects for fibrillation or fasciculation of skeletal musculature during or after testing, the doses of anti-ChEs used in relation to the route of application are considered unlikely to have induced muscle damage in most of the subjects. Even if a subacute neuromyopathy had occurred in a few subjects, especially those who reportedly displayed weakness and muscle twitching after exposure, experience with laboratory animals suggests that these changes would have been resolved within weeks.

Although some of the OP agents tested can induce peripheral neuropathy in laboratory animals, the doses needed to induce clinical signs greatly exceed the LD₅₀ for that species (105, 112, 113). Humans are generally understood to develop neuropathy after receiving doses comparable with those which induce clinical signs in hens (69, 121); because the doses used on the test subjects were far below those needed to induce experimental neuropathy, it is most unlikely that any subject experienced delayed onset of distal limb paralysis. However, clinical signs of neuropathy in experimental animals occur some time after the onset of CNS-PNS distal axonopathy, after a particular number of nerve fibers have undergone degeneration. It is therefore not possible to rule out the chance that subclinical pathologic changes occurred in vulnerable nerve-fiber pathways in subjects treated with agents known to be capable of inducing neuropathy. PNS nerve fibers probably undergo regeneration and re-establishment of end-organ connection promptly after the degenerative phase has terminated; damaged CNS fibers are unlikely to regenerate functional connections, but minor damage of this type usually does not induce noticeable functional impairment. Development in the test subjects of anything more than minor and transient sensorimotor manifestations as an expression of such putative damage is considered most unlikely and no such symptoms were recorded. Neurologic examination would reveal signs of long-term CNS changes, such as the Babinski sign (extensor plantar response), hyperactive ankle or knee jerk, and spastic or ataxic gait. Such signs would be permanent attributes of a person who suffered this type of CNS damage, but such changes in the test subjects are considered unlikely.

OTHER ADVERSE HEALTH EFFECTS

Mutagenicity, Carcinogenicity, and Male Reproductive Effects

There is little information on mutagenicity, carcinogenicity, and male reproductive effects, in relation to anti-ChE chemicals. Experimental evidence suggests that malathion does not pose a mutagenic or carcinogenic risk to exposed humans, nevertheless, some scientists disagree with this conclusion (157). The safety of the other compounds could not be confidently determined, because of the absence of laboratory studies. Nevertheless, the Panel is unaware of any reports linking these adverse health effects to single or repeated exposure to anti-ChE agents. Furthermore, there is no suggestion of increased mortality from carcinogenicity, although final judgment on this issue must be reserved until mortality and morbidity data are collected and analyzed.

Information on whether the tested subjects have sired children and on the state of health of their offspring since testing would be helpful in evaluating the possibility of anti-ChE-induced mutagenicity or adverse effects on the male reproductive system.